fects of dynamic mechanical compression on prostaglandin E_2 (PGE_2) production by the knee joint meniscus and to determine whether this response was dependent on the magnitude of applied stress.
Methods: Cylindrical meniscus explants (5 mm diameter, 2 mm thick) were harvested from knee joints of 2-year-old female pigs. Specimens were obtained from the outer one-third ("outer zone") and the inner two-thirds ("inner zone") of the femoral surface of the medial meniscus, which did not show any degenerative changes or tears. Following preincubation for 72 hours, compressive loads were applied using a modified version of the Biopress system (Flexcell International). Dynamic mechanical compression was performed at 0.5 Hz at a compressive stress of 0.0125, 0.025, 0.05, 0.1, 0.2 or 0.5 MPa for 24 hours. For each level of stress investigated, 12 samples from the outer zone and eight samples from the inner zone (N=4 joints) were used and the site-matched control explants maintained unloaded. PGE_2 production was measured using an ELISA kit (R&D Systems). In addition, meniscal explants were tested in unconfined compression in a closed-loop, force-controlled testing system (ELF 3200, EnduraTEC) in order to calculate the mean deformation of the explants at each stress level.
Results: Dynamic mechanical compression of meniscal explants significantly increased PGE_2 production, which was maximal at 0.2 MPa and a drop-off at 0.5 MPa. Compression significantly stimulated PGE_2 production at most magnitudes of stress for inner zone meniscal explants ($P<0.001$ at 0.05–0.2 MPa, $P<0.01$ at 0.5 MPa) and all magnitudes of stress for outer zone meniscal explants ($P<0.01$ at 0.0125 MPa, $P<0.001$ at 0.025–0.2 MPa, $P<0.05$ at 0.5 MPa). At 0.2 MPa, a significant difference between inner and outer zone explants was observed ($P<0.001$), demonstrating a higher increase in PGE_2 production in the inner zone.
Discussion: Our results suggest that the meniscus may serve as a potent contributor to PGE_2 production in the knee joint. Previous studies have shown that osteoarthritic menisci also show significant increases in PGE_2 synthesis in response to various pro-inflammatory cytokines. Similar to articular cartilage, the production of PGE_2 was dependent on the magnitude of stress. Furthermore, differences were observed in the production of PGE_2 with site, with greater PGE_2 production in the inner zone as compared to the outer at the same stress magnitudes. These findings suggest that, in addition to the articular cartilage, the menisci may contribute to the presence of pro-inflammatory mediators in the knee joint, and thus may provide an additional target for pharmaceutical intervention in the treatment arthritis.
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P213

DOWN REGULATION OF DEGENERATIVE MOLECULES IN OSTEOARTHRITIC CHONDROCYTES GROWN ONTO A HYALURONAN SCAFFOLD

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Aim of the study: To investigate whether a hyaluronic-based biomaterial, already used as scaffold for autologous chondrocyte transplantation, could create also an environment which favours the downregulation of catabolic factors expression.
Methods: Human chondrocytes were isolated from articular cartilage obtained from patients with early-onset knee osteoarthritis. The cells were expanded in monolayers and then seeded on a hyaluronic-acid derivative scaffold. Constructs and surnatants were collected and analyzed at 1, 3, 7, 14 and 21 days after seeding. Immunohistochemical analysis for CD44 and caspase was carried out on paraffin embedded sections. The Tunel method was used to identify chondrocyte apoptosis status. Secretion of MMP-1 and MMP-13 in the surnatants of the cells grown onto the biomaterial was measured by enzyme-linked immunosorbent assay. Nitric oxide (NO) production was evaluated by estimating the stable NO metabolite nitrite by the Griess method. A Real-Time RT-PCR analysis was performed on the constructs to evaluate the expression of type I and II collagens, aggrecan, Sox-9, MMP-1 and MMP-13 mRNAs at the different experimental times evaluated.
Results: The presence of CD 44 receptor increased over time showing the highest positivity at day 21. Immunohistochemistry for caspase-3 displayed many positive cells at 1 day after seeding of chondrocytes onto the biomaterial. This positivity decreased during the culture period and on day 21 the cells were almost negative. Decreased levels of metalloproteinases and nitric oxide were observed in the surnatants of chondrocytes grown onto the hyaluronan-based scaffold. This was also confirmed by Real-Time PCR analysis which showed that the cells expressed the specific differentiated phenotype and in the meantime downregulate the expression of some catabolic molecules. Cells apoptosis decreased during the culture period, further supporting the biochemical data.
Conclusions. The hyaluronan-based biomaterial used in this study acts on chondrocyte metabolism downregulating catabolic pathways. The ability to reduce the expression and production of molecules involved in cartilage degenerative processes by oasteoarthritis chondrocytes indicates its use also in the transplantation therapeutical strategy to treat early lesions in these patients.

P214

HYDROSTATIC LOADING EFFECTS ON A SUSPENSION-CULTURED CARTILAGE TISSUE EQUIVALENT

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Aim of the Study: The objective of this study was to determine if cyclical hydrostatic pressure, applied to a unique suspension culture method that forms cartilage tissue equivalents (CTE), altered or improved their characteristics.
Methods: Eight 1-week-old CTE were divided into two groups. The CTE were generated using 10^8 neonatal porcine chondrocytes/ml grown in 24-well dishes coated with poly-HEMA to prevent attachment. Four specimens serve as controls and the remaining four were hydrostatically pressurized. The wells were vacuum-sealed in polyurethane bags and placed in a custom designed pressure chamber. The device pressurized the chamber and the CTE through loading with a materials testing machine.Cyclical loading from 0.5-5 MPa was applied for 3 hours, three days a week at a frequency of 0.1 Hz. Following the completion of each loading session, specimens were removed from the vacuum sealed wells, placed in storage wells with fresh medium, and returned to the incubator. This process was repeated for 3 consecutive weeks. Upon completion specimens were tested mechanically and biochemically and compared with control. One dimensional confined compression incremental stress-relaxation tests were performed on five-millimeter diameter plugs. An initial tare load of 0.08 N was then applied and then five 7.5% compressive strain steps were conducted at a rate of 2.5 N/m-s followed by a 1400-second hold. The resulting stress initially peaked and then relaxed to an equilibrium value during the displacement hold.

The aggregate modulus (Hao) and the permeability (k_o) was obtained from a biphasic model. Collagen was analyzed by pepsin digestion, SDS-PAGE, and western blotting and proteoglycan by GAG dye-binding assay. Studies are being performed to analyze the mRNA for constitutive cartilage genes using real-time PCR.
Results: The aggregate modulus of the control and pressurized specimens was 15.1 kPa (0.86 SD) and 21.1 kPa (3.85 SD), respectively, an increase of 39.7%. The permeability decreased from $10.52x10^{-13}$ m^4/Ns ($9.4x10^{-13}$ SD) for the control specimens to $1.27x10^{-13}$ m^4/Ns ($0.56x10^{-13}$ SD) for the pressurized specimens. Medium was found to contain increasing collagen type II (CII) and no type I. GAG assays performed on the medium show a 4-fold increase by 2 wk where a 3-fold increase was found in the pressure treated cultures after 3 wks. Thus far the increase of CII and PG is indicative of an increase in biosynthetic activity.
Conclusions: These results signify that the application of hydrostatic pressure improved the mechanical properties of the CTE. Additional studies testing CTE loaded for varying times and doses will optimize the material properties and suitability for use in cartilage repair. The values of aggregate modulus that were obtained in this study fall within the range of those seen in other tissue engineering studies.

P215

MEASUREMENT OF THE THREE-DIMENSIONAL STRAIN AND DIFFUSION COEFFICIENT IN ARTICULAR CARTILAGE UNDER COMPRESSION

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Aim of the Study: Diffusion facilitates molecular transport in articular cartilage and is affected by mechanical loading is essential. Methods will be developed to characterize the relationship between diffusion and strain on the cellular scale whihc may aid in understanding the development of osteoarthritis and serve as a reference to researchers attempting to grow tissue engineered cartilage.
Methods: A method was developed to mechanically load a murine humeral head while imaging the loaded cartilage using a multi-photon/confocal microscope. Coupled measurements of molecule diffusion and strain were collected. A linear slide (Parker Hannifin Corporation, Irwin, PA) was driven with a stepper motor (Lin Engineering, Santa Clara, CA) and moved a sensor assembly consisting of a load cell, a linear transducer, a specimen holder and a small piece of tubing to surround the specimen. The sample was compressed by pushing it into the cover slip on the inverted microscope (Carl Zeiss LSM 510 NLO, Thornwood, NY). Fluorescence recovery after photobleaching (FRAP) measured the rate of diffusion of fluorescent dextrans (10 kDa) in the extracellular matrix. A 22 micron diameter region, 5.0 microns from the cartilage surface was bleached with the laser and the recovery observed as fluorescent molecules diffused back into the bleached region. The diffusion constant was then calculated from theory. The 3D strain field was simultaneously measured by tracking stained chondrocyte nucleui. The z-stack data was processed and nuclei volumes detected and meshed. The displacement field within the volume was calculated using image cross correlation and a Lagrangian finite strain was determined. Data was collected at the same location at three static compressive loads, approximately 0 N and 0.05 N, and 0.1 N.
Results: The relationship between molecule diffusion and compressive load was found. There is an initial decrease in diffusion rate, then an increase in diffusion rate with applied load near 0.015 N compression. The relationship between diffusion and first principal strain is plotted in Fig. 1 and shows the diffusion rate decreases slightly until approximately 0.2 strain, then increases with larger strain values.

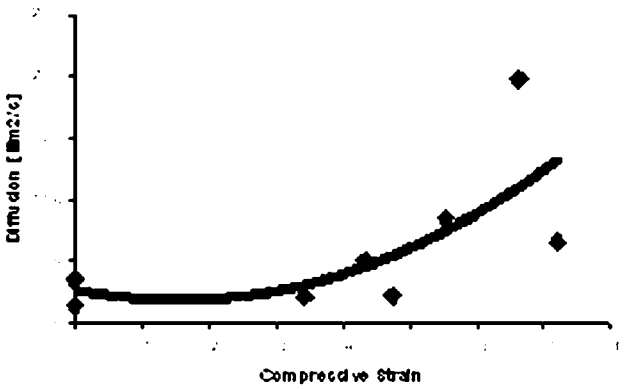

Fig. 1. Diffusion constant vs. compressive strain relationship.

Conclusions: A stationary point in the diffusivity-strain curve was present where diffusivity begins to increase with load and compressive strain. We hypothesize that during the strains from 0-20%, diffusivity decreases as the matrix volume decreases and then as strain increases, collagen fibers that are randomly oriented may become ordered due to stronger interactions among charged proteoglycans, and resulting in changes in the diffusivity. Future work should investigate this behavior.

P216

VALIDATION OF A RABBIT MODEL OF POSTEROLATERAL KNEE INSTABILITY

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Posterolateral knee instability is a difficult clinical problem to diagnose and treat. Development of an appropriate animal model of this condition would be useful in evaluating its natural history and the effects of surgical interventions. The goals of the present study were to validate a rabbit model of posterolateral knee instability to determine: 1) if instability persists six months after injury and 2) if it leads to the development of osteoarthritis. The fibular collateral ligament and popliteus tendon were surgically transected in one knee in each of ten skeletally mature New Zealand white rabbits, with the contralateral knee serving as a control. The rabbits were euthanized at six months postoperatively and the knees were removed and tested biomechanically for joint stability. The articular cartilage of each proximal tibia and distal femur was then evaluated grossly (India ink), midsagittal histological sections of each proximal tibia were graded using an osteoarthritis grading scheme, and articular cartilage and subchondral bone thicknesses and areas were measured. The lesions were most severe in the medial tibial plateau in all joints; therefore, histological grading was confined to this site. Biomechanical testing revealed a statistically significant difference in the amount of rotation of the operated knees compared to the unoperated control knees to varus moments at 30°, 60°, and 90°, and to external rotation torques at 30° and 60° (Table 1).
There were no significant differences in internal rotation motion or valgus displacement between the operated and unoperated control knees at any flexion angle. Morphological analysis (India ink staining, histology of HE and toluidine blue stained sections, and histomorphometry) revealed a trend toward more severe lesions of osteoarthritis in the medial compartment of the operated knees compared with the unoperated knees, although these changes were not statistically significant. This study demonstrates that untreated posterolateral knee injuries in rabbits have not healed six